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(12) **PATENT APPLICATION A1**

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(60) References to other related domestic documents:

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(54) USE OF A PEPTIDE PREVENTING INTOLERANCE REACTIONS OF THE SKIN, ESPECIALLY IN COSMETIC COMPOSITIONS.

(57) Use as additives, in cosmetic compositions, of a peptide containing the sequence Lysine-Proline-Valine, for the purpose of preventing or reducing the intolerance reactions associated with contact hypersensitivity.

The invention concerns a method of cosmetic treatment enabling, in particular, prevention or reduction of intolerance reactions of the skin, thanks to the use of a cosmetic composition containing a specific peptide as an additive.

The use of such a peptide as an additive in a cosmetic composition enables reducing the risks of contact dermatitides (also called contact eczemas) or other skin reactions resulting from contact hypersensitivity.

It is known that contact hypersensitivity reactions are provoked by small molecular weight allergens (haptens) which are capable of bonding to the proteins present in epidermal cells, including the proteins of antigen-presenting cells (Langerhans' cells), to form protein-hapten complexes (or conjugates). The antigen-presenting cells of the epidermis then migrate and sensitize the T lymphocytes. After a latency period of approximately 4 to 5 days, when an epidermal site is once again in contact with the allergen (triggering reaction), the sensitized T-cells migrate to this site and cause the release of cytokines which provoke the arrival of nonsensitized T-cells and macrophages, which are in turn activated. This afflux of cells can, in particular, translate into the formation of edema or of vesicles usually occurring after a few hours, i.e., 1 or 2 days after the triggering reaction, in individuals already sensitized.

Contrary to other allergic reactions, provoked by an excessive reduction of immunoglobulins IgE, contact hypersensitivity reactions are primarily associated with a cellular immunologic response. Contact hypersensitivity is linked by immunologists to type IV delayed hypersensitivity, also called cell-mediated hypersensitivity.

The sensitizing substances may be quite diverse. They are, in particular, metals or metallic salts (chrome, nickel, cobalt, mercury), formol, synthetic resins (epoxy resins, acrylic and phenolic resins), latex, locally applied medications, certain cosmetics, dyes or dye precursors (paraphenylenediamine, aniline, amino phenols), pesticides, certain synthetic fabrics, certain vegetable products (in particular, pentadecylcatechols, or "poison ivy"), or preservatives. Thus, numerous substances are likely to provoke contact dermatitides, both in professional life and in everyday life.

Contact hypersensitivity, which manifests itself, in particular, by an irritation, may, in its acute forms, provoke erythema, edema, vesicle formation, or pruritus or a burning sensation.

The most severe forms of contact dermatitides require pharmaceutical treatment by corticosteroids. Consequently, these are used subsequent to a triggering of the hypersensitivity reaction. Their therapeutic use after the fact, although efficacious, is thus curative and not preventive. Moreover, their mode of action may provoke adverse effects over the long term (in particular, bleaching of the skin, atrophy of the skin, and risk of cutaneous superinfection).

In addition, it is known that a great many molecules, of great diversity, are likely to be used as constituents of cosmetic compositions. It is also known that an increase in the number of cases of cross contact hypersensitivity reactions is currently being noted. Thus, the application of cosmetic compositions entails the risk of such reactions in users.

Consequently, it is desirable to have new agents available enabling prevention of contact hypersensitivity reactions or reduction of the risks of the occurrence of such a reaction in humans, especially in individuals with hypersensitive skin, also referred to as intolerant and/or reactive skin.

The present invention is based on the discovery of properties of certain peptides capable of preventing contact hypersensitivity reactions or reducing the risks of occurrence of such a reaction. Such an effect is clearly different from an anti-inflammatory effect, as is demonstrated, for example, by the experimental section below.

The invention concerns more precisely the use of specific peptides containing the peptide sequence Lysine-Proline-Valine as additives in a cosmetic composition or as active ingredients in the preparation of a medication designed to suppress or reduce contact hypersensitivity reactions, with the exception of the use of melanotropin. Melanotropin, also called ∀-MSH, is a known natural peptide with 13 amino acid residues (all of L configuration) and containing the peptide sequence indicated at positions 11 through 13.

Activity of melanotropin as a modulator of the contact hypersensitivity response was described by RHEINS et al., J. Invest. Dermatol. 93, 511-517 (1989).

Each of the amino acid residues of the peptides used according to the present invention may be of either configuration, L or D.

Thus, the object of the invention is, in particular, a cosmetic treatment method characterized in that in order to prevent or to reduce the intolerance reactions of the skin associated with contact

hypersensitivity, an ordinary cosmetic composition containing, as an additive, at least one peptide containing the peptide sequence Lysine-Proline-Valine, with the exception of melanotropin, or a derivative of such a peptide is applied to the skin or to the exoskeleton.

The term "exoskeleton" refers to the hair, the nails, and the eyebrows and eyelashes.

The cosmetic treatment method of the invention is intended, in particular, for individuals with hypersensitive or intolerant skin.

A further object of the invention is the use as an active ingredient, in the preparation of a medication designed to combat (preventively or curatively) contact hypersensitivity reactions, of at least one peptide (or of a derivative of such a peptide) containing the peptide sequence Lysine-Proline-Valine, as defined above, with the exception of the use of melanotropin.

The peptide used according to the invention may contain, in particular, from 3 to 10 amino acid residues, in particular, 3, 4, 5, or 6 amino acid residues, each of which may independently have L or D configuration.

By way of example, at least one amino acid residue of the peptide, in particular at least one amino acid residue of the peptide sequence Lysine-Proline-Valine, and especially the proline residue, may have the D configuration. The invention includes the use of peptides such as those defined above in which the amino acid residues constituting the peptide sequence Lysine-Proline-Valine all have the D configuration. The peptides used according to the invention are, in particular, those in which, moreover, at least one part (and possibly all) of the amino acid residues other than those of the Lys-Pro-Val sequence has the D configuration.

The invention concerns, in particular, the use of peptides containing at least the peptide sequence of formula I:

Lys-Pro-Val (I)

as well as the use of derivatives of these peptides, with the exception of the use of melanotropin. The derivatives of the peptides containing the sequence of formula I are, for example, those of which at least one functional group (in particular the amine and carboxyl groups) is protected by a protecting group. Of course, the protecting group must be compatible with use in the cosmetic or pharmaceutical field. The common protecting groups are well known. The peptide derivatives include, in particular, those for which the terminal carboxyl group and the other carboxyl groups possibly present are in the form of an ester (for example, a low alkyl ester) or of

an amide, and/or for which the N-terminal amine group and the other amine groups possibly present are in an acylated form (for example, acetylated).

Among the peptides usable according to the invention, specifically mentioned are those in which the Val residue in the Lys-Pro-Val sequence constitutes the C-terminus of this sequence, and especially those for which the Lys-Pro-Val sequence constitutes the C-terminus of the peptide (with the C-terminal amino acid being Val). Mentioned by way of example are the peptides which contain at least one of the following tripeptide sequences: D-Lys-D-Pro-D-Val, D-Lys-D-Pro-L-Val, L-Lys-D-Pro-D-Val, or L-Lys-D-Pro-L-Val. The tripeptides constituted by the sequences just mentioned are called peptides of formula Ia, Ib, Ic, and Id, respectively.

The derivative of the peptide of the formula Ia of which the lysine residue is acetylated and whose carboxyl group of the valine residue is amidated is represented by the formula II:

Ac-D-Lys-D-Pro-D-Val-NH_2 (II)

The peptides used according to the invention and their derivatives can be prepared according to the usual techniques of peptide synthesis.

The compositions containing the peptide used according to the invention can be administered orally, parenterally, or topically.

To that end, they can be presented, in particular, in the form of tablets, capsules, drinkable solutions, injectable solutions, lyophilizates for injectable solutions, lotions, gels, or liquid or semi-solid emulsions.

Such compositions contain the peptide used according to the invention at concentrations which may vary generally from 10^{-12} M to 10^{-2} M, and specifically from 10^{-7} M to 10^{-3} M.

These compositions are prepared according to the usual methods.

The pharmaceutical compositions obtained according to the invention contain, besides the active ingredient, and possibly associated with other active ingredients, at least one appropriate pharmaceutical vehicle.

The invention also concerns the use of at least one peptide or peptide derivative, as defined above, in cosmetic compositions (in particular, compositions for the skin, for the nails, or for the

hair), as an additive designed to suppress or to reduce the risks of contact hypersensitivity reactions for the users of the compositions. It is a question of reducing both the risks of a reaction to potentially sensitizing external agents and the risks of a reaction to substances present in the cosmetic composition which constitute potential allergens.

The cosmetic compositions thus obtained contain, besides the peptide, the usual ingredients and vehicles present in the type of composition in question. These compositions are, in particular, lotions, creams, or gels for the face, the neck, or the hands, lipsticks, makeup compositions, makeup removers or skin cleansers, mascaras, makeup foundations, face powders, soaps, perfumes, nail polishes, or hair compositions, such as, shampoos, hair lotions, hair coloring compositions, permanent wave compositions, etc.

The interest of the presence of a peptide used according to the invention in compositions for hair obviously results from the fact that at the time of their use, these compositions are most often in contact with the scalp, resulting in a risk of contact allergy. In the case of compositions for nails, the interest is that the nails are often in contact with the skin.

In the following experimental section, the abbreviation TNCB stands for trinitrochlorobenzene.

PHARMACOLOGICAL STUDY

1) Study of the effect of the peptide of formula II on the response to sensitization by TNCB, in mice

a) *Reduction of the contact hypersensitivity reaction after sensitization*

The study was performed on female mice Balb/C, 7 to 10 weeks old.

A dose of 1.5 µg of the peptide under investigation (in solution in PBS buffer containing 0.1 % mouse serum) is injected into a vein of the tail of the mouse 2 hours before the sensitization reaction.

The sensitization reaction consists in applying by brush 100 µl of a 0.15-% solution of TNCB in a mixture of acetone-olive oil (4 : 1) to the shaved skin of the abdomen of the mice.

The triggering reaction is carried out 7 days after the sensitization reaction. This triggering reaction consists in applying, on both sides of one ear, 10 microliters of a 0.8-% solution of TNCB in a vehicle made up of the acetone-olive oil mixture already mentioned above.

On the other ear, for comparison, this mixture of acetone and olive oil alone is applied.

The intensity of any contact hypersensitivity reaction is evaluated by estimating the magnitude of the edema of the ear treated with the TNCB compared with the ear treated with the vehicle alone. For this, the thickness of the ears is measured using a micrometer screw. This measurement is made 24 hours after the triggering reaction.

TNCB is also applied to the ear of control mice not sensitized in advance (negative controls).

The positive controls do not receive the peptide before the sensitization reaction.

Results

In this experiment, the increase in the thickness of the ear in the positive controls is on average

9.8×10^{-2} mm. In the negative controls, this increase is on the order of 1×10^{-2} mm. For the animals treated with the peptide under investigation, this increase is only approximately 2.5×10^{-2} mm.

These results demonstrate that the peptide under investigation greatly reduces the contact hypersensitivity reaction.

b) *Reduction of the contact hypersensitivity reaction after a second sensitization*

Fourteen days after the end of the experiment described in a) above, the same mice are subjected to a new sensitization reaction on the shaved skin of the back, as described in a).

Seven days later, the triggering test is performed by application of TNCB on the ear; and 24 hours later, the level of swelling of the ear is measured.

The object of this second experiment is to make a distinction between possible temporary aspecific immunosuppression and specific immunological tolerance.

Results

For the positive controls, the average increase in ear thickness is on the order of 14×10^{-2} mm. For the animals which had been treated with the peptide II in the first experiment, just as for the negative controls, the level of swelling of the ear is on the order of 3.5×10^{-2} mm.

These results demonstrate that the animals treated with the peptide under investigation before the first sensitization did not develop a contact hypersensitivity after application of a second sensitizing dose of TNCB. The mice thus treated, consequently, became tolerant to TNCB.

c) *Effect of the peptide of formula II in topical application*

The same procedure is followed as in a) above; however, the peptide, incorporated in a cream of the water-in-oil type, is applied to the zone to be sensitized 2 hours before the sensitization.

Twenty-four hours after the triggering reaction, the increase in ear thickness is on average 3×10^{-2} mm in the treated mice and 7×10^{-2} mm in the positive controls.

2) Study of the effect with topical application in humans

This study was performed on volunteer patients 18 through 65 years of age, in whom a contact allergy to nickel was suspected. These patients were free of other skin disorders, and the results of this study demonstrate that they had no allergy to the constituents of the cream used as the vehicle.

On day 0, on the one hand, a cream containing 100 µM of the peptide of formula II and, on the other, the cream alone, without peptide, used as a placebo, is applied with an occlusive dressing to zones of the skin of the back.
Two hours later, patches impregnated with vaseline containing nickel sulfate at a concentration of 5 % are applied to the same zones of the skin and also on an adjacent zone not pretreated.

Twenty-four hours later, the patients are examined. Those who developed a contact hypersensitivity reaction on the zone treated by the placebo (and also on the adjacent zone which received only the application of nickel salt), and who are consequently individuals who were already sensitized to nickel, numbered 14. Of these 14 individuals, 7 developed no contact

hypersensitivity reaction at all on the zone treated by the peptide.

These results demonstrate that the peptide under investigation acts locally, on the application site, since the adjacent cutaneous zone which received only the application of nickel salt had a contact hypersensitivity reaction.

EXAMPLES OF COMPOSITIONS

EXAMPLE 1: *Lotion for the scalp*

- Peptide of formula II ..... 12.5×10^{-6} g
- 2, 4-diaminopyrimidine-3-oxide ..... 0.75 g
- Ethanol at 95 % ..... 30 g
- Perfume ..... qs
- Dyes ..... qs
- Demineralized water ..... qsp 100 g

EXAMPLE 2: *Skin care cream* (oil-in-water emulsion)

- Mixture (80 : 20) of cetylstearylic alcohol and cetylstearylic alcohol oxyethylenated with 33 moles of oxyethylene ..... 5 g
- Glycerol monostearate ....1.5 g
- Cetylic alcohol .....0.75 g
- Vaseline oil .....10 g
- Polydimethylsiloxane .....0.75 g
- Glycerin .....4 g
- Preservatives..... qs
- Peptide of formula II .....5 mg
- Demineralized water .....qsp 100 g

EXAMPLE 3: *Intradermally injectable solution*

- Peptide of formula II .....0.7 mg
- Apyrogenic sterile aqueous solution with 9 % NaCl .....qsp 1 ml

CLAIMS

1. Method of cosmetic treatment, characterized in that, to prevent or to reduce intolerance reactions of the skin associated with a contact hypersensitivity, a common cosmetic composition containing, as an additive, at least one peptide or peptide derivative containing the peptide sequence Lysine-Proline-Valine, with the exception of melanotropin, is applied to the skin or to the exoskeleton.

2. Method according to claim 1, wherein the peptide contains from 3 to 10 amino acid residues.

3. Method according to any one of the preceding claims, wherein the peptide used contains from 3 to 6 amino acid residues.

4. Method according to any one of the preceding claims, wherein at least one of the amino acid residues, specifically the proline residue, of said peptide sequence has the D configuration.

5. Method according to any one of the preceding claims, wherein the amino acid residues constituting the Lysine-Proline-Valine peptide sequence all have the D configuration.

6. Method according to any one of the preceding claims, wherein all the amino acid residues of the peptide have the D configuration.

7. Method according to any one of the preceding claims, wherein said peptide derivative is selected from among the peptides of which at least one functional group is protected by a protecting group.

8. Method according to any one of the preceding claims, wherein said the additive is the tripeptide D-Lys-D-Pro-D-Val or a derivative thereof.

9. Use of at least one peptide containing the peptide sequence Lysine-Proline-Valine, or at least one derivative of such a peptide, in cosmetic compositions, as an additive designed to suppress or to reduce the risks of contact hypersensitivity reactions, with the exception of the use of melanotropin.

10. Use according to claim 9, wherein said peptide or peptide derivative is as defined in any one of claims 2 through 8.

11. Use as the active ingredient, in the preparation of a medication designed to combat contact hypersensitivity reactions, of at least one peptide containing the peptide sequence Lysine-Proline-Valine, or of at least one derivative of such a peptide, with the exception of the use of melanotropin.

12. Use according to claim 11, wherein said peptide or peptide derivative is as defined in any one of claims 2 through 8.

FRENCH REPUBLIC --- NATIONAL INSTITUTE OF INDUSTRIAL PROPERTY	**PRELIMINARY SEARCH REPORT** established on the basis of the latest claims filed prior to the beginning of the search	**2784028** National registration no: FA 565120 FR 9812300

DOCUMENTS CONSIDERED RELEVANT		Pertinent claims of the application examined
Category	Citation of the document with indication, as needed, of the relevant parts	

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X	WO 88 00833 A (UNIV TEXAS) February 11, 1988 (02/11/1988) *entire document* ---	1-12
X	US 4,874,744 A (NORDLUND JAMES J ET AL.) October 17, 1989 (10/17/1989) *entire document* ---	1-12
D, X	L. A. RHEINS: "Alpha-Melanocyte Stimulating Hormone Modulates Contact Hypersensitivity Responsiveness in C57/BL6 Mice" THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 93, no. 4, October 1989 (10/1989), pp. 511-517, XP002109102 *entire document* ---	1,9,11
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TECHNICAL FIELDS SEARCHED (Int. Cl. 6)
A61K C07K

Date search terminated	Examiner
July 14, 1999	Sierra Gonzalez, M

CATEGORY OF THE DOCUMENTS CITED

X: particularly relevant by itself
Y: particularly relevant in combination with another document of the same category
A: pertinent with regard to at least one claim or general technical background
O: unwritten disclosure
P: interleaved document

T: theory or principle basic to the invention
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